

Amendments to the Claims

1-19 (Canceled)

20. (Currently amended) A plasmid having a nucleic acid sequence region comprising an open reading frame encoding a cleavable single-chain polypeptide, said open reading frame comprising:

- a) a first nucleotide sequence region comprising
 - i) a first portion encoding a first amino acid sequence region comprising a binding element able to specifically bind a target cell surface marker under physiological conditions; and
 - ii) a second portion encoding a second amino acid sequence region comprising a translocation element able to facilitate the transfer of a polypeptide across a vesicular membrane;
- b) a second nucleotide sequence region encoding a third amino acid sequence region comprising a therapeutic element having biological activity when released into the cytoplasm of the target cell, and

wherein said first and second nucleotide sequence regions are separated by a third nucleotide sequence region encoding a fourth amino acid sequence comprising a protease cleavage site which is cleaved when exposed to a protease, provided said third amino acid sequence region is not cleaved by a human protease or a protease is not normally expressed by a cell expressing said single-chain polypeptide, and wherein said single-chain polypeptide is expressed by said plasmid within a suitable host cell.

21. (Original) The plasmid of claim 20 wherein said first or second nucleotide sequence region further encodes an amino acid sequence region comprising a binding tag.

22. (Original) The plasmid of claim 21 wherein said binding tag comprises a target-binding portion of a polypeptide selected from the group consisting of His₆, monoclonal antibodies, maltose binding protein, glutathione-S-transferase, protein A, and calmodulin binding protein.

23. (Original) The plasmid of claim 20 wherein said first nucleotide sequence region encodes at least a portion of a clostridial neurotoxin heavy chain.

24. (Original) The plasmid of claim 23 wherein said first nucleotide sequence region encodes at least a portion of a *Clostridium botulinum* neurotoxin heavy chain.

25. (Original) The plasmid of claim 23 wherein said first nucleotide sequence region encodes at least a portion of a *Clostridium tetani* neurotoxin heavy chain.

26. (Currently amended) The plasmid of either of claim[s] 20 or 23 wherein said second nucleotide sequence region encodes at least a portion of a clostridial neurotoxin light chain.

27. (Original) The plasmid of claim 26 wherein said second nucleotide sequence region encodes at least a portion of a *Clostridium botulinum* neurotoxin light chain.
28. (Original) The plasmid of claim 26 wherein said second nucleotide sequence region encodes at least a portion of a *Clostridium tetani* neurotoxin light chain.
29. (Original) The plasmid of claim 20 wherein said first nucleotide sequence region encodes a binding element which will specifically bind a cell type other than a motor neuron.
30. (Original) The plasmid of claim 29 wherein said first nucleotide sequence region encodes a binding element that will specifically bind a cell type selected from the group consisting of a pancreatic acinar cell and a sensory afferent neuron.
31. (Original) The plasmid of claim 20 wherein said second nucleotide sequence region encodes a therapeutic element other than a clostridial neurotoxin light chain.
32. (Original) A method of making a single-chain polypeptide derived from a clostridial neurotoxin comprising:
- a) inserting the plasmid of any one of claims 20-25 or 29-31 into a suitable host cell,
 - b) growing said host cell in culture, and
 - c) permitting or inducing the host cell to express the single chain polypeptide encoded by said plasmid.
33. (Currently amended) A method of purifying ~~the~~ a recombinant single chain polypeptide of ~~claim 13 derived from a clostridial neurotoxin comprising the steps:~~
- a) lysing a cell expressing a single chain polypeptide from the plasmid of either of claim 21 or 22 to produce a cell lysate, and
 - b) contacting said cell lysate with a target compound so as to form a specific binding complex capable of being immobilized comprising said binding tag and said target compound[.], and
 - c.) separating said binding complex from said cell lysate.
34. (Canceled)
35. (New) A method of making a single-chain polypeptide derived from a clostridial neurotoxin comprising:
- a) inserting the plasmid of claim 26 into a suitable host cell,
 - b) growing said host cell in culture, and
 - c) permitting or inducing the host cell to express the single chain polypeptide encoded by said plasmid.
36. (New) A method according to claim 35, wherein said plasmid is of claim 27.
37. (New) A method according to claim 35, wherein said plasmid is of claim 28.